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A diversity oriented synthesis of natural product inspired molecular libraries

Jyoti Chauhan,[a] Tania Luthra,[a] Rambabu Gundla[b], Antonio Ferraro,[c] Ulrike Holzgrabe[c] and Subhabrata Sen[a,] *

[a] Department of Chemistry, School of Natural Sciences, Shiv Nadar University, Dadri, Chithera, GautamBudh Nagar, UP 201314, India

subhabrata.sen@snu.edu.in

[b] Department of Chemistry, Gitam Institute of Technology, GITAM University, Hyderabad, Telengana, India

[c]Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany

Abstract

Natural products are the origin for innumerable pharmaceutical drug candidates and also forms an important aspect of herbal remedies. They are source of various bioactive compounds. Herein we have leveraged structural attributes of several natural products in building a library of architecturally diverse chiral molecules by harnessing R-tryptophan, as the chiral auxiliary. It is converted to its corresponding methyl ester **1** which in turn provided a bevy of 1-aryl-tetrahydro-β-carbolines **2a-d** which were then converted to chiral compounds *via* diversity oriented synthetic strategy (DOS). In general intermolecular and intramolecular ring rearrangements facilitated the formation of the final compounds. Four different classes of molecules with distinct architecture were generated accounting to nearly twenty two individual molecules. Phenotypic screening of a representative section of the library revealed two molecules that selectively inhibit MCF7 breast cancer cells with IC_{50} of ~5 μgm/mL potency.

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Introduction

To investigate complex biological system, small molecules have been applied to modulate proteins by directly interacting with them.[1] In conventional genetic approaches biological systems were assessed by developing random mutations which were then screened in search of a precise cellular phenotype.[2] Analogous to the genetic approach, large random collections of small molecules can be used to elucidate the roles of specific proteins in many biological pathways.[3] The essence of this chemical genetic approach is the design and synthesis of a library of compounds which cover large areas of biologically relevant chemical space.[4]

By virtue of binding both their biosynthetic enzymes and their target molecules, natural products necessarily reside in biologically relevant chemical space.[5] Natural product families are libraries of prevalidated, functionally diverse structures.[6] Therefore developing library of molecules with scaffolds inspired from natural products could provide biologically active novel molecules.[7] There are quite a few reports of library of molecules inspired from natural products.[8a-d] For example, Khan *et al.* reported synthesis of a molecular library inspired from marine natural product ianthelliformisamines and their biological evaluation, in another report, Singh and co-workers generated a natural product inspired β-carboline and γ-lactones based molecular hybrids and Waldmann reported stereoselective synthesis of natural product inspired tetrahydroindolo[*2, 3-a*]-quinolizine compound library.[8c-d]

Additionally, to counter the challenge of gaining rapid access to structurally diverse natural product inspired libraries, diversity oriented synthesis (DOS) is an ideal tool.[9] This concept discovered by Schreiber at the beginning of last decade, is a forward directional strategy that involves facile preparation of libraries of architecturally complex and diverse compounds from simple starting materials. DOS can be achieved through various approaches.[10] One such strategy, the reagent based approach, involves generation of a densely functionalized central building block which in turn can be transformed into various scaffolds by subjecting them to varied tranformations.[11] Oxidative rearrangement chemistry extends interesting possibilities for DOS application, especially in reagent based approaches. In this direction a bevy of diverse reactions can be performed to generate scaffolds with diverse architecture.[12]

Here in we have reported the design and synthesis of a library of molecules based on natural product Perophoramidine, Spirotryptostatin, Harmicin and Tryprostatin A and B (Figure 1a).[13]

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The resulting compounds were generated by modifying a central tetrahydro-β-carboline moiety under various reaction conditions which underwent amalgamation of the scaffolds of the aforementioned natural products to generate **3**, **4**, **5** and **6** (Scheme 1b). Such an effort is purely intuitive with the expectation that the process will provide novel biologically active molecules. In general, this strategy involved a DOS, *via* a novel (oxidative) inter/ intramolecular ring rearrangement. A representative section of the library was screened against MCF7 cell lines to assess their ability to modulate a particular cancer phenotype.

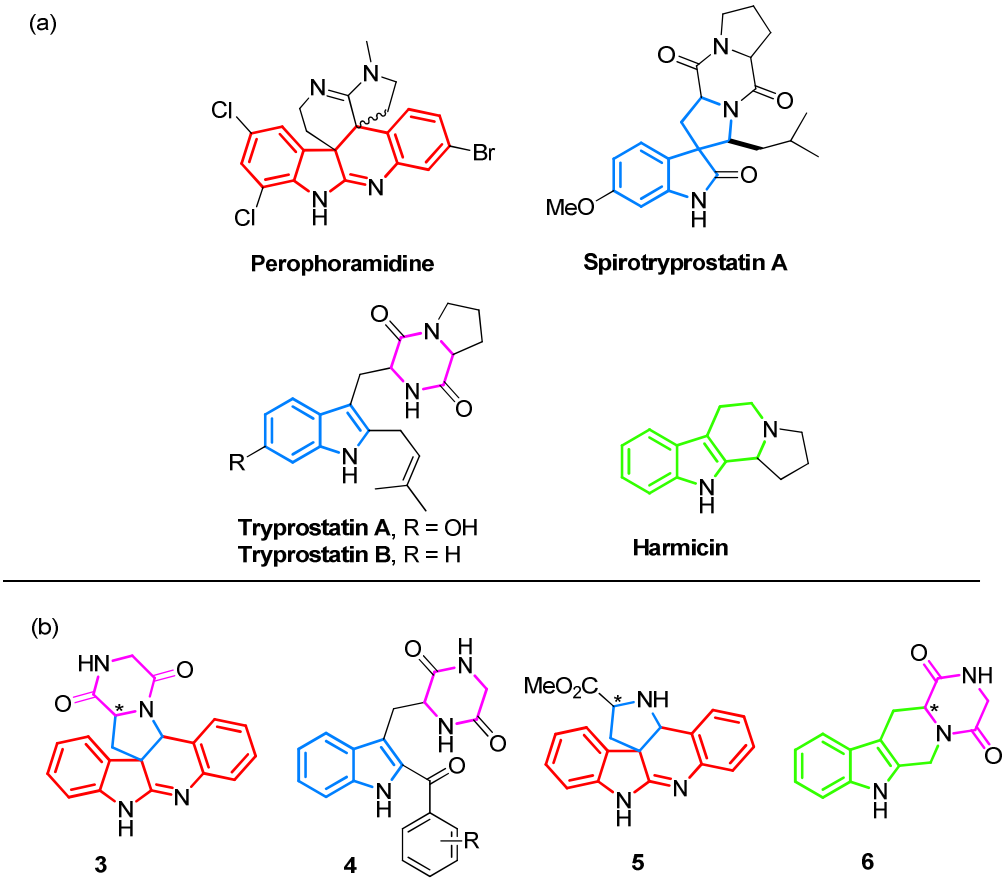

Figure 1. Natural products and the library inspired from them

Results and discussion

Design

The design of this library is based on tetrahydro-β-carbolines **2a-d**. They were conceived as central scaffolds because they contained several pluripotent reaction centers such as a, b, c, d and e (Scheme1). Careful choice of reaction condition will enable us to harness these centers

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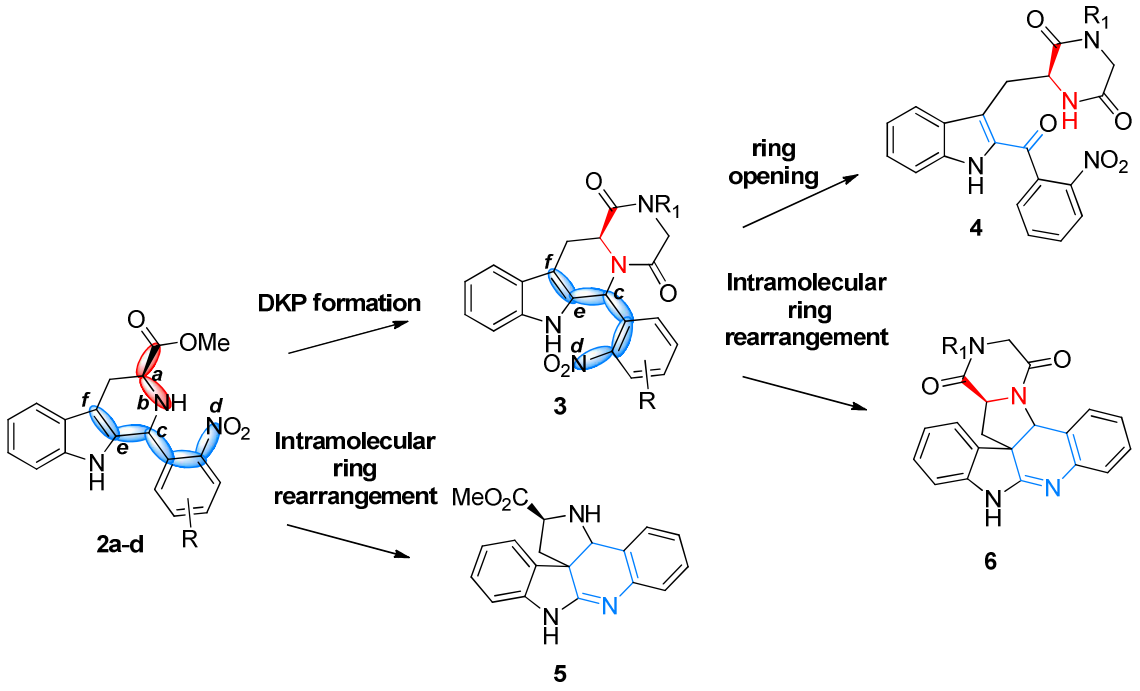
selectively to provide us the desired scaffolds **3**-**6**. The design enabled the library to proliferate from one scaffold to the other. For example, compound **2**, could provide **3** and **5**. **3** in turn could furnish **4** and **6** (Scheme 1). Accordingly, we envisioned that compounds **3**, could be accessed by exploiting the reactivity of center a and b of **2**, by sequential treatment of chloroacetyl chloride and appropriate amines (Scheme 1). Next, compound **4** could be obtained from **3** by an oxidative ring opening reaction involving centers c, e and f (Scheme 1). We further envisioned that **6** could also be accessed from **3** *via* an intramolecular ring rearrangement involving centers c, d, e and f (Scheme 1). A similar oxidative ring rearrangement of **2** again involving centers c-f could also provide compound **5** (Scheme 1).



Scheme 1. Design of the hybrid library

Chemistry

With the design in place we embarked on the synthesis of the molecular library. To begin with, the central tetrahydro-β-carboline building blocks **2a-d** were synthesized *via* a Pictet Spengler[13f] reaction between **1** (obtained by methylation of R-tryptophan) and appropriately substituted *o*-nitrobenzaldehydes (i.e. *o*-nitrobenzaldehyde, 4-chlorobenzaldehyde, 5-chlorobenzaldehyde and veratraldehyde) (Scheme 2). A typical reaction involved refluxing **1**, 1.5 equiv. of trifluoroacetic acid and 1.2 equiv. of *o*-nitrobenzaldehyde in acetonitrile. The desired products were obtained as

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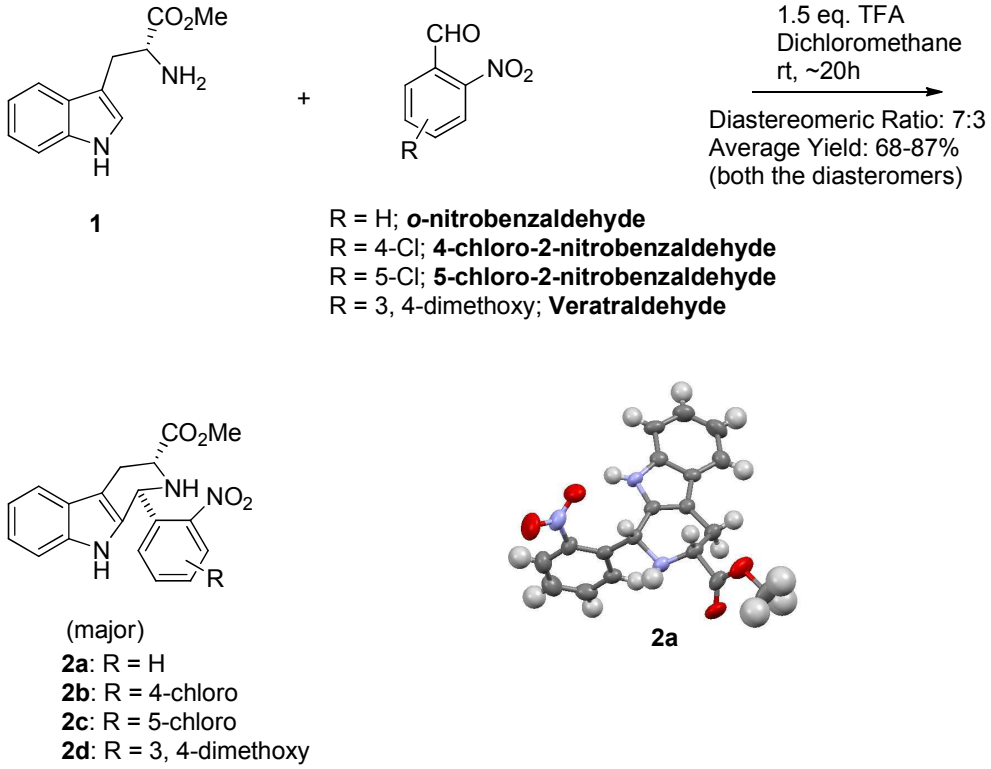
1:1 diasteromeric mixtures. From the desired products that where generated we opted to proceed with the compound **2a** (in a bid to prove this modular concept this was a random choice) which was isolated *via* column chromatography. Accordingly, single crystal X-ray of **2a** (refer *SI*) indicated that, the methyl ester and the aryl moiety were in syn conformation. Compounds **2b-d** were generated in a similar fashion and were taken forward to proliferate the library (Scheme 2).



Scheme 2. Synthesis of central scaffolds **2a-d**

The first set of molecules **3a-g**, that we envisioned, possessed tetrahydro-β-carboline diketopiperazine (DKP) scaffolds. Accordingly **2a**, was treated with chloroacetyl chloride to provide amide **9a** which was further treated with methyl amine to facilitate an *in situ* nucleophilic substitution followed by lactamization(with the ester) to generate the desired hybrid **3a** in 58% yield. In a similar fashion, **2a**, after reaction with chloroacetyl chloride ($ClCH_2COCl$) was treated with various other amines i.e. ethyl, benzyl, hexyl, propyl and isopropyl to afford **3b-f**. Accordingly **2d** provided **3h**.

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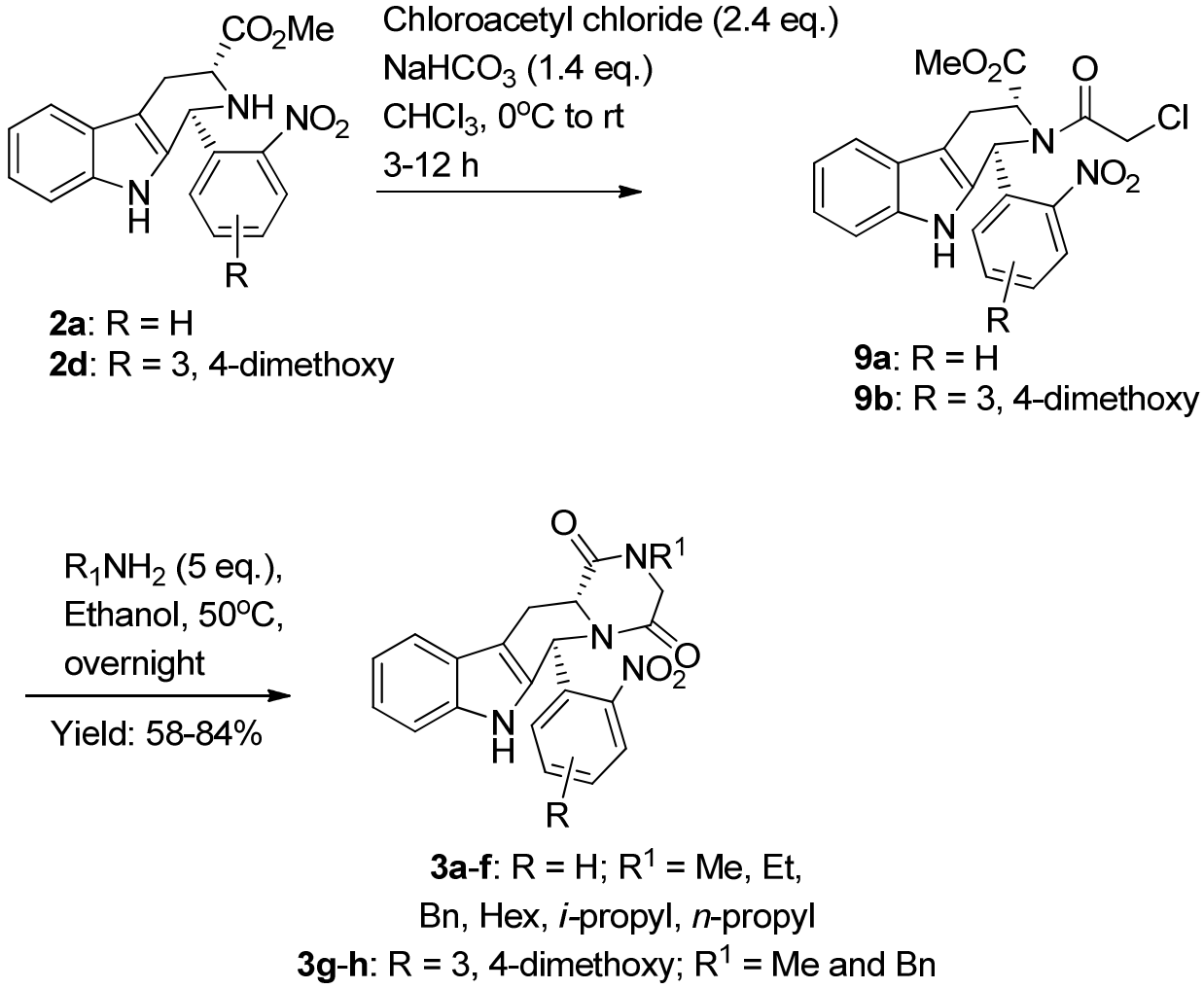

Scheme 3. Synthesis of tetrahydro-β-carboline-DKP hybrids **3a-g**

Next, as envisioned during the design of the molecules, sodium dithionate mediated nitro reduction of **3a-d**, **f** and **g** facilitated the amine intermediates **10a-d**, **f** and **g** (Scheme 4). The reduction was extremely facile with no formation of any by-products. Consequently, amines **10a-d**, **f** and **g**, were taken to the next step without further purification. To promote the intramolecular oxidative ring rearrangement of these amines, variety of oxidants such as *t*-butoxychloride (*t*-BuOCl), N-bromosuccinimide (NBS), sodium tungstate (dehydrate) ($Na_2WO_4.2H_2O$), lead tetraacetate($Pb(OAc)_4$) and osmium tetraoxide (OsO_4)were explored with **10a** as the model substrate. To our extreme gratification, NBS furnished the best yield (~91%) of the desired hybrid **6a** which is the major diastereomer in a 70:30 mixture of the products. It was separated

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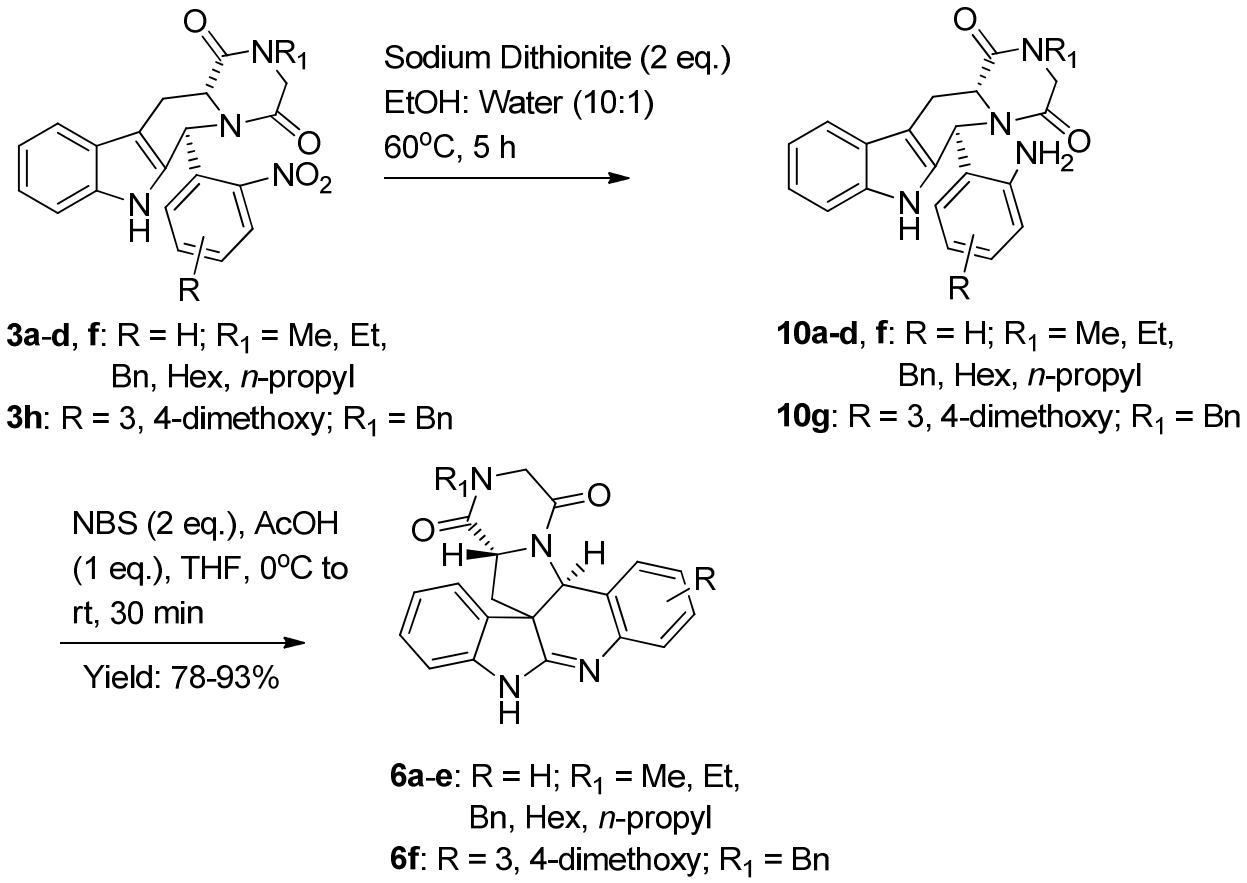
from the mixture by column chromatography. With the optimized condition in hand, the other hybrids, **6b-f** were also synthesized in moderate to excellent yield (Scheme 4).



Scheme 4. Synthesis of hybrids **6a-f**

The rational of transformation of the resulting hybrids **6a-f** from the corresponding amine intermediate could be explained by the putative mechanism of the intramolecular ring rearrangement of **6f**, as depicted in Scheme 5 below. The reaction involved treatment of **10f** with NBS as an oxidant, which brominates at C_X of **10f** to generate **A**. The bromination is expected to occur from the β face to avoid the steric interaction from the DKP and 2-nitroaryl

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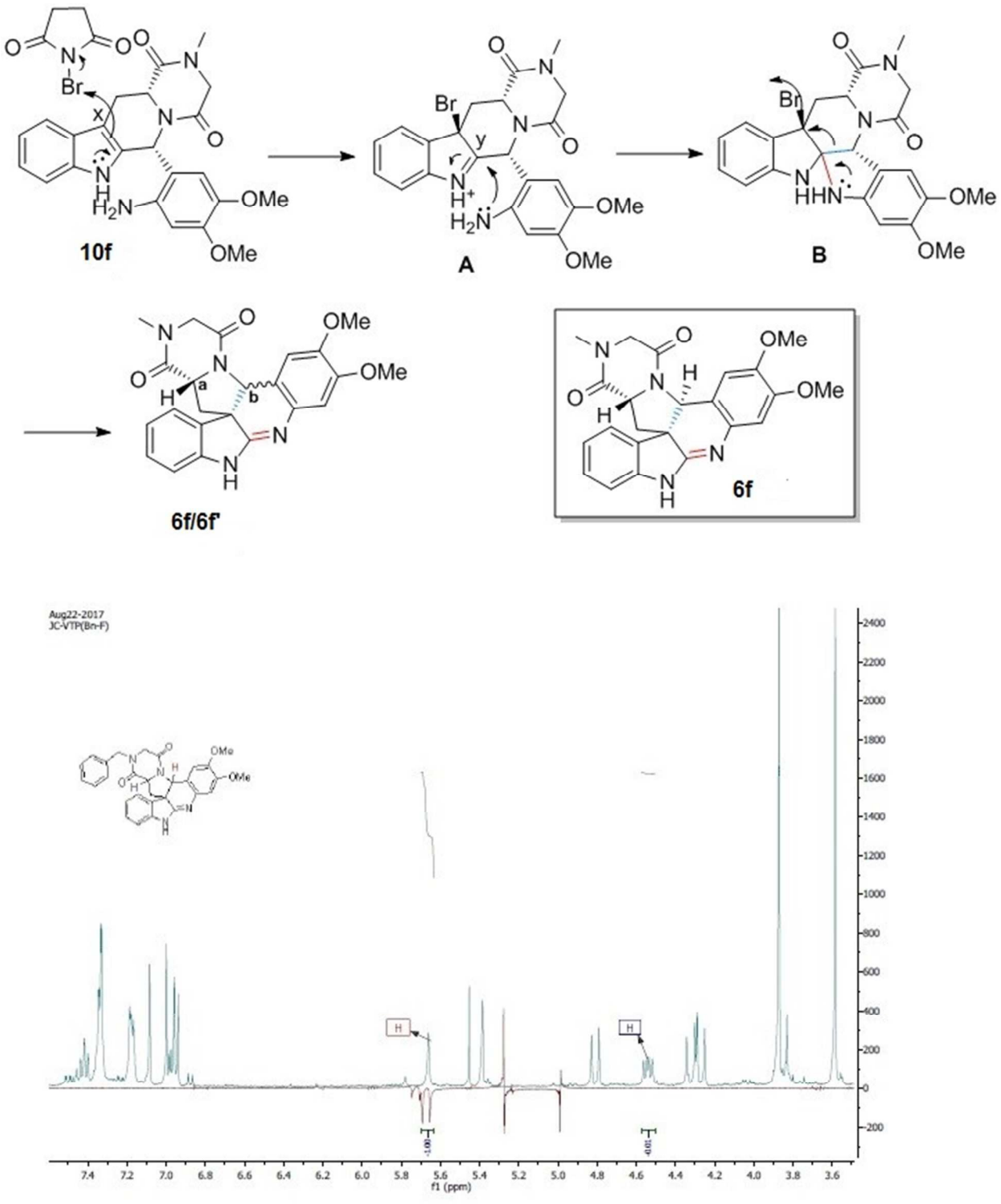

Scheme 5. Putative mechanism of the intramolecular ring rearrangement to generate **6**

moiety. A nucleophilic attack by the aromatic amine at C_Y generated **B** followed by a final bond rearrangement prompted by the imine formation, where the bond shift happened from the

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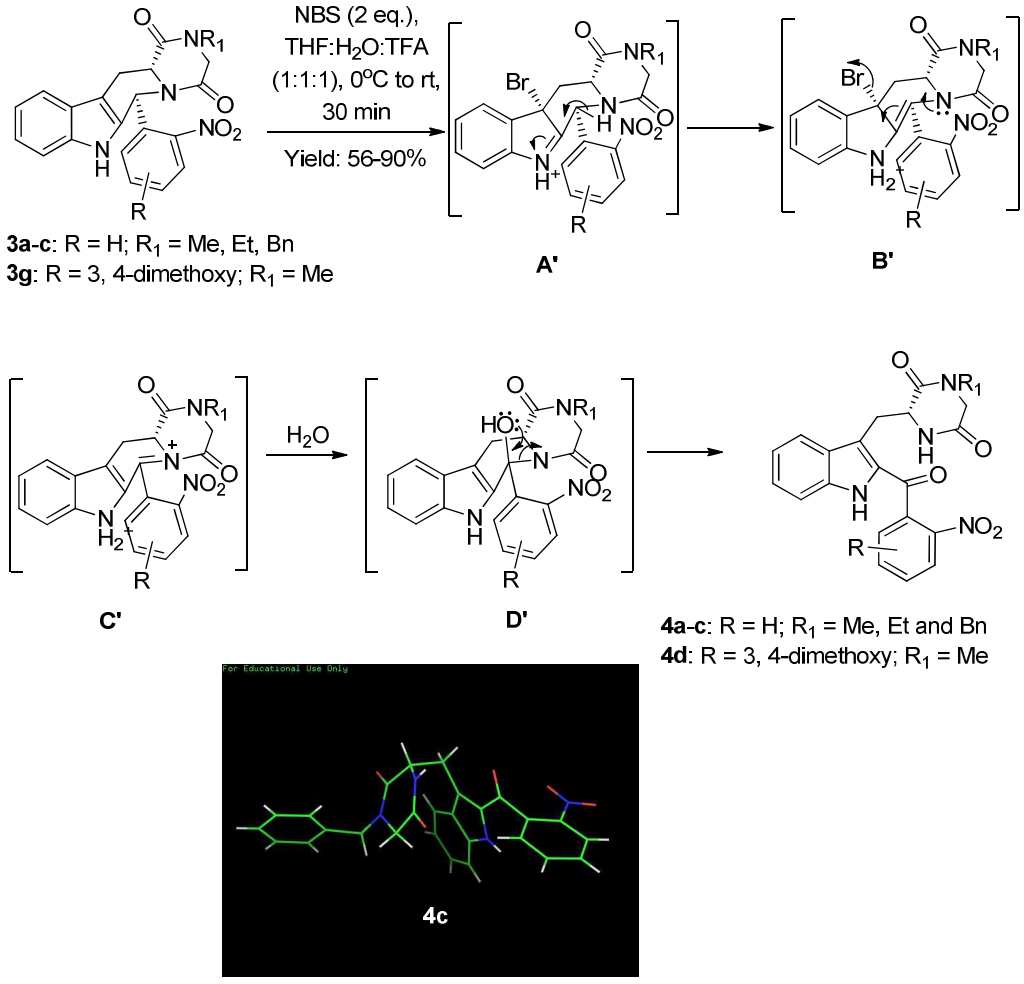
opposite face of the bromide functionality (in a fashion similar to S_{N2} reactions) to afford the desired products **6f**/**f´** as 70:30 mixture of diastereomers (Scheme 5) from which the major diastereomer, **6f** was isolated by column chromatography). 1D-NOESY experiment of **6f** provided the relative configuration of the stereocenters **a** and **b** as S and R respectively (Scheme 5).

In a bid to further diversify our scaffold library, **3a-g** were treated with NBS in presence of tetrahydrofuran: water: trifluoroacetic acid (1:1:1). Initially the bromination occurred at C_3 of the indole skeleton to afford the bromo iminium intermediate **A´**, which subsequently leads to the formation of enamine **B´**. Elimination of the bromide on **B´**generated **C´** followed by the attack of the water molecule to afford hemiaminal **D´**. **D´** then underwent ring opening to furnish the desired hybrids **4** (scheme 6).



Scheme 6. Synthesis of hybrid **4a-g**

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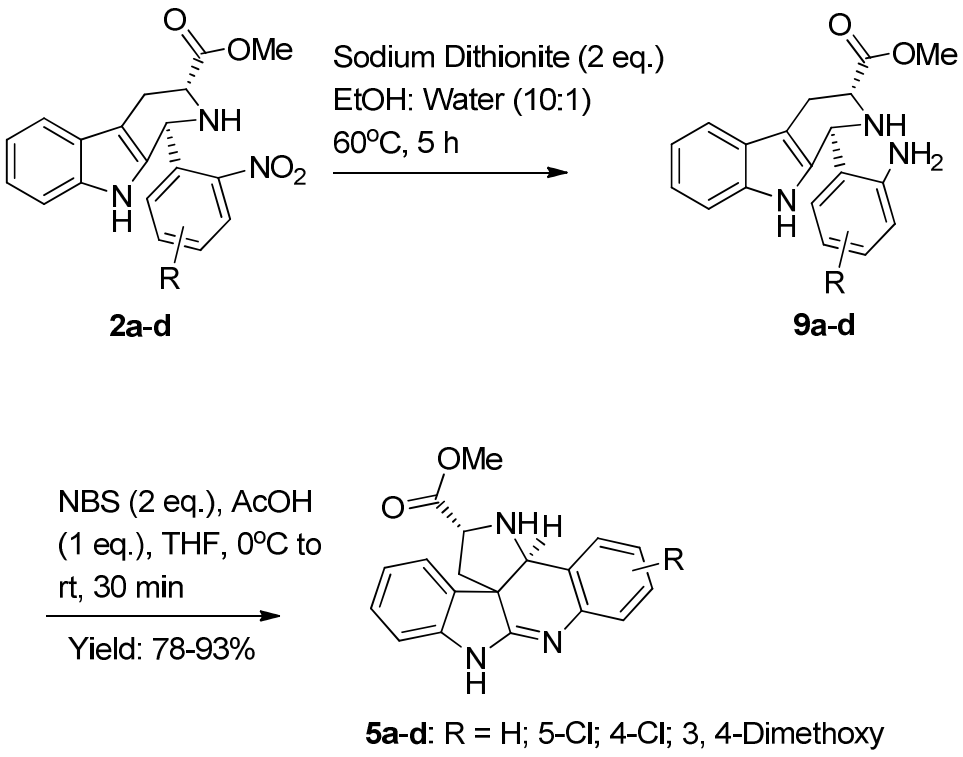
Finally compounds **5a-d** were synthesized *via* a similar ring rearrangement as in compounds **6a-g** from the starting substrate **2a-d**, by their subsequent reduction with sodium dithionite (to generate **9a-d**) followed by treatment with NBS and acetic acid. Here too we obtained the major diastereomers **5a-d** from a 80:20 mixture, by column chromatography in 60-72% yield (Scheme 7).



2a-d **9a-d**

Scheme 7. Synthesis of hybrid scaffolds **5**

Phenotyping screening

The original natural products that inspired our molecules have a rich legacy as anticancer compounds. Subsequently we decided to screen our molecules for anticancer activity. Due to the paucity of the cell lines, a representative selection of the library of molecules were screened against MCF7 (breast cancer cells; Table 1). Potent cancer drug Doxorubicin was used as the positive control. From the screening it was evident that our library of molecules was efficacious against MCF7 cells (Table 1). Among the compounds screened, **6a** and **6e**, (comprised of indoloquinolone, spirooxindole and DKP scaffolds) inhibited the proliferation of MCF7 cells with an IC_{50} of ~5 μgm/mL. To investigate whether our compounds deploy their anticancer activity through cytotoxicity, we screened them against MCF10A, a normal

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cell line. For **6a** and **6e**, the dose required to induce cytotoxicity was ~100 fold higher than their respective IC_{50} concentration (Table 1). These anticancer hits at low μgm/mL level, fluently accentuates the merit of our DOS library, since the presumption from such unbiased, early scaffold exploration are modest (double digit μgm/mL potency). This further underlines the "significance" of the hybrid aspect of our library and the power of the DOS approach to deliver hit and sometimes lead structures.

Table 1. Inhibition of proliferation of MCF7 cancer cells

Entry	Compound #	IC_{50} (μgm/mL)[a] MCF7
1	**3a**	16.28
2	**3b**	9.21
3	**3e**	12.88
4	**3f**	9.21
5	**4g**	15.95
6	**6a**	5.01
7	**6b**	9.58
8	**6c**	7.02
9	**6d**	8.32
10	**6e**	5.33
11	**6f**	14.01
12	**5a**	28.91
13	**Doxorubicin**	1.81

[a]The results showed are expressed as mean ± SEM of three independent assays.

Conclusion

We have reported a DOS strategy based primarily on oxidative ring rearrangement and ring opening reactions to synthesize a library of chiral molecules with high architectural diversity. An extremely versatile tetrahydro-β-carboline motif was harnessed as the central building block. The molecules inspired from diverse natural products are essentially comprised of four distinct architectures. This modular strategy demonstrated an efficient 2 steps/scaffold ratio. Phenotypic

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screening against MCF7 breast cancer cells displayed selective low μmolar inhibitory activity by two compounds against MCF7 cell proliferation. This effort demonstrates the utility of an unbiased approach like diversity oriented synthesis in discovering novel bioactive scaffolds. The low μmolar hits against MCF7 obtained from this endeavor can be utilized further to develop drug candidates for breast cancer through protein deconvolution and subsequent structure activity relationship studies.

EXPERIMENTAL SECTION

General. All reactions were carried out under N_2 or O_2 atmosphere as specified. Column chromatography was performed on Silica gel (100-200 mesh), and reactions was monitored by thin layer chromatography (TLC, Silica gel 60 F_{254}), using UV light to visualize the course of the reaction. ^{1}H NMR and ^{13}C NMR spectra were recorded with tetramethylsilane as an internal standard at ambient temperature unless otherwise indicated with Bruker 400 MHz instruments at 400 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR spectroscopy. Splitting patterns are designated as singlet (s), broad singlet (br. s), doublet (d), triplet (t). Splitting patterns that could not be interpreted or easily visualized are designated as multiplet (m). Mass spectrometry analysis was done with a 6540 UHD Accurate-Mass QTOF LC–MS system (Agilent Technologies) equipped with an Agilent 1290 LC system obtained by the Department of Chemistry, School of Natural Sciences, Shiv Nadar University, Uttar Pradesh 201314, India.HPLC experiments were carried out in Agilent Eclipse Plus C18 column. SEM and EDX analysis was carried out in Zeiss EVO 18 Special system. ICP-MS experiment was carried in Element XR system. TEM analysis was carried out in Tecnai G2 F30 (300kV) system.

Representative experimental procedures for Pictet Spengler reactions of R-trytptophan and appropriate aldehydes (2a-d). To the solution of L-tryptophan methyl ester in dichloromethane (DCM) was added substituted 2-nitrobenzaldehydes with trifluoroacetic acid (TFA) and allowed to stir at room temperature for 24 h. After completion of reaction, reaction mixture was concentrated under reduced pressure and quenched with saturated solution of sodium bicarbonate ($NaHCO_3$). It was then extracted with ethyl acetate (EtOAc) (3 X 20 mL). The organic layer was washed with brine solution and dried over sodium sulphate (Na_2SO_4) and concentrated over reduced pressure to obtain crude product. It is purified with column chromatography.

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(1R, 3S)-methyl-1-(2-nitrophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate (2a):Following the general procedure the desired compound **2a** was generated as yellow solid with yield of 89%(purified *via* flash column chromatography with ethyl acetate-hexane (2:8) as eluent).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.86 (dd, *J* = 1.2, 8 Hz, 1H), 7.73 (dd, *J*= 1.2, 8 Hz, 1H), 7.57-7.50 (m, 2H), 7.44 (m, 1H), 7.60-7.23 (m, 1H), 7.18-7.11 (m, 2H), 5.71 (s, 1H), 3.96 (dd, *J*= 4, 10.8 Hz,1H), 3.83 (s, 3H), 3.27 (dd, *J*= 2.4, 15.2 Hz, 1H), 3.06 (m, 1H), 2.95 (s, 1H) ;^{13}C NMR (100 MHz, $CDCl_3$) δ 172.92, 150.26, 136.45, 133.61, 133.40, 131.80, 129.03, 126.88, 123.77, 122.36, 119.86, 118.45, 111.17, 109.66, 56.58, 53.18, 52.48, 25.49. HRMS (EI+) m/z calcd. for $C_{19}H_{17}N_3O_4$ $[M]^+$: 352.1292, found: 352.1291.

(1R,3S)-methyl-1-(4-chloro-2-nitrophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate (2b):Following the general procedure the desired compound **2b** was generated as light yellow solid with yield of 79% (purified *via* flash column chromatography with ethyl acetate-hexane (1:9) as eluent).^{1}H NMR (400 MHz; $CDCl_3$): 8.21 (s, 1H); 7.79-7.78 (d, *J*= 4 Hz, 1H); 7.54-7.52 (d, *J*= 8 Hz, 1H); 7.50-7.48 (m, 2H); 7.24-7.15 (m, 3H); 5.94 (s, 1H); 4.09-4.05 (m, 1H); 3.83 (s, 3H); 3.31-3.26 (s, 1H); 3.17-3.10 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): 169.38, 149.62, 137.13, 136.88, 134.09, 133.63, 128.49, 126.20, 125.73, 125.58, 123.54, 120.35, 118.56, 111.70, 108.62, 53.32, 52.80, 50.57, 22.30. $[M+H]^+$ calculated for ($C_{19}H_{16}N_3ClO_4$) 386.0902, found 386.0928

(1R,3S)-methyl-1-(5-chloro-2-nitrophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3-carboxylate (2c):Following the general procedure the desired compound **2c** was generated as light yellow solid with yield of 72% (purified *via* flash column chromatography with ethyl acetate-hexane (1:9) as eluent).^{1}H NMR (400 MHz; $CDCl_3$): 8.24 (s,1H); 7.68-7.65 (d, *J*= 12 Hz, 2H); 7.54-7.52 (d, *J*= 8 Hz,1H); 7.35-7.28 (m, 2H); 7.24-7.16 (m, 2H); 6.12 (s, 1H); 4.14-4.09 (m, 1H); 3.87 (s, 3H); 3.36-3.31 (s, 1H); 3.25-3.19 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): 169.68, 148.03, 140.30, 136.81, 130.72, 126.22, 125.81, 123.21, 120.34, 118.44, 117.38, 111.80, 108.77, 65.88, 55.86, 53.27, 23.04.$[M+H]^+$ calculated for ($C_{19}H_{16}N_3ClO_4$) 386.0902, found 386.0929

Methyl-1-(4,5-dimethoxy-2-nitrophenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole-3 carboxylate (2d):Following the general procedure the desired compound **2d** was generated as yellow colored solid with yield of 87% (purified *via* flash column chromatography with ethyl

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acetate-hexane (3:7) as eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.48 (s, 1H), 7.28 (s, 1H), 7.22 (s, 1H), 7.20-7.13 (m, 2H), 5.86 (s, 1H), 4.01 (d, *J* = 3.6 Hz, 1H), 3.99 (d, *J* = 4 Hz, 1H), 3.96 (s, 3H), 3.86 (s, 3H), 3.80 (s, 3H), 3.30-3.25 (m, 1H), 3.12-3.05 (m, 1H) ;^{13}C NMR (100 MHz, $CDCl_3$) δ 172.99, 153.56, 148.55, 142.52, 136.38, 133.80, 131.48, 126.97, 122.25, 119.78, 118.38, 112.10, 111.19, 109.42, 107.05, 56.68, 56.56, 56.53, 53.34, 52.47, 25.38. HRMS (EI+) m/z calcd. for $C_{21}H_{21}N_3O_6$ $[M]^+$: 412.1503, found: 412.1532.

Representative experimental procedures for the synthesis of tetrahydro-β-carboline-diketopiperazine hybrids (3a-h). To the stirred solution of compound **2a** and **d** (1 equiv.) and sodium bicarbonate ($NaHCO_3$) (1.2 equiv.) in chloroform ($CHCl_3$) was added dropwise chloroacetyl chloride (2.4 equiv.) at 0 ºC. The reaction mixture was stirred at room temperature under nitrogen atmosphere for 3 h. After completion of reaction which was monitored by TLC the reaction mixture was diluted by $CHCl_3$ and washed with saturated solution of $NaHCO_3$, brine, dried over Na_2SO_4, and evaporated under reduced pressure to obtained crude product which was taken to the next step without purification.

To the solution of the above crude compound (1 equiv.) and appropriate amine (5 equiv.) in ethanol was stir at room temperature for 24 h. It was then concentrated under reduced pressure after completion of reaction. The crude product was purified with column chromatography with ethyl acetate-hexane as eluent.

2-methyl-6-(2-nitrophenyl)-2,3,12,12a-tetrahydropyrazino[1', 2': 1,6]pyrido[3,4-b]indole-1, 4(6H, 7H)-dione (3a):Following the general procedure the desired compound **3a** was generated as light yellow solid with yield of 58% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (3:7) as eluent). ^{1}H NMR (400 MHz, DMSO) δ 10.53 (s, 1H), 7.96 (d, *J* = 7.2 Hz, 1H), 7.58 (d, *J* = 8 Hz, 1H), 7.50 (t, 1H), 7.43-7.39 (m, 1H), 7.33 (s, 1H), 7.31 (s, 1H), 7.06 (t, *J* = 6.8, 16 Hz, 1H), 7.01 (t, *J* = 7.2, 14 Hz, 1H), 6.71 (s, 1H), 4.50 (d, *J* = 7.2 Hz, 1H), 4.01 (s, 1H), 3.91 (s, 1H), 3.63-3.59 (m, 1H), 3.18 (m, 1H), 2.87 (s, 3H) ; ^{13}C NMR (100 MHz, DMSO) δ167.15, 165.70, 148.82, 138.23, 137.01, 133.81, 131.87, 128.91, 127.98, 125.70, 12359, 121.62, 118.98, 118.19, 111.82, 106.00, 55.55, 51.52, 50.91, 32.69, 24.08, 18.55. HRMS (EI+) m/z calcd. for $C_{21}H_{18}N_4O_4$ $[M]^+$: 391.1401, found: 391.1403.

2-ethyl-6-(2-nitrophenyl)-2,3,12,12a-tetrahydropyrazino[1', 2': 1, 6]pyrido[3,4-b]indole-1, 4(6H, 7H)-dione (3b): Following the general procedure the desired compound **3b** was generated

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as light brown solid with yield of 62% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (3:7) as eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 7.85 (d, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.44 (t, *J* = 7.2, 14.8 Hz, 1H), 7.33 (s, 1H), 7.32 (s, 1H), 7.30 (s, 1H), 7.21-7.13 (m 2H), 6.715 (s, 1H), 4.37 (dd, *J* = 3.6, 11.3 Hz, 1H), 4.12 (d, *J* = 7.2 Hz, 1H), 4.07 (d, *J* = 17.2 Hz, 1H), 3.86 (s, 1H), 3.83 (t, *J* = 4.4, 8 Hz, 1H), 3.70 (q, *J* = 6.8, 13.6 Hz, 1H), 3.39-3.32 (m, 2H), 1.19 (t, *J* = 7.2, 4.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.78, 165.58, 149.27, 138.33, 136.54, 133.88, 131.45, 128.22, 127.98, 126.15, 124.07, 123.00, 120.23, 118.77, 111.50, 106.72, 60.54, 56.36, 52.36, 52.48, 49.37, 41.44, 24.48, 14.34, 12.09. HRMS (EI+) m/z calcd. for $C_{22}H_{20}N_4O_4$ $[M]^+$: 381.1557, found: 381.1570. M. P. 98 °C

2-benzyl-6-(2-nitrophenyl)-2,3,12,12a-tetrahydropyrazino[1', 2': 1, 6]pyrido[3, 4-b]indole-1, 4(6H, 7H)-dione (3c): Following the general procedure the desired compound **3c** was generated as grey solid with yield of 56% over two (purified *via* flash column chromatography with ethyl acetate-hexane (2:8) as eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.84 (d, *J* = 8 Hz, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 7.44 (t, *J* = 7.6, 14.8 Hz, 1H), 7.34-7.27 (m, 8H), 7.19 (t, *J* = 6.8, 14.4 Hz, 1H), 7.16 (t, *J* = 5.2, 12.8 Hz, 1H), 4.89 (d, *J* = 14.4 Hz, 1H), 4.89 (d, *J* = 14.4 Hz, 1H), 4.41 (d, *J* = 4 Hz, 1H), 4.12 (q, *J* = 7.2 Hz, 1H), 3.93 (d, *J* = 5.2 Hz, 1H), 3.89 (d, *J* = 4.4 Hz, 1H), 3.79 (d, *J* = 18 Hz, 1H), 3.38 (dd, *J* = 11.6, 16 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.54, 166.07, 149.19, 138.34, 136.51, 135.11, 133.87, 131.43, 129.16, 128.40, 128.23, 127.89, 126.12, 124.12, 123.01, 120.24, 118.74, 111.51, 106.63, 60.54, 56.34, 52.45, 49.96, 49.41, 24.59, 21.20, 14.34. HRMS (EI+) m/z calcd. for $C_{27}H_{22}N_4O_4$ $[M]^+$: 467.1714, found: 467.1830.

2-hexyl-6-(2-nitrophenyl)-2,3,12,12a-tetrahydropyrazino[1', 2': 1, 6]pyrido[3, 4-b]indole-1, 4(6H, 7H)-dione (3d): Following the general procedure the desired compound **3c** was generated as grey solid with yield of 77% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (3:7) as eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.85 (d, *J* = 8 Hz, 1H), 7.61 (d, *J* = 8 Hz, 1H), 7.42 (d, *J* = 8 Hz, 1H), 7.33-7.30 (m, 3H), 7.21-7.130 (m, 2H), 6.72 (s, 1H), 4.38 (dd, *J* = 4.4, 11.2 Hz, 1H), 4.12 (d, *J* = 8 Hz, 1H), 4.06 (d, *J* = 8 Hz, 1H), 3.86 (s, 1H), 3.81 (s, 1H), 3.61-3.54 (m, 1H), 3.38-3.29 (m, 1H), 2.04 (s, 1H), 1.59 (s, 3H), 1.30 (s, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.84, 165.85, 149.25, 138.40, 136.54, 133.87, 131.44, 128.22, 127.95, 126.16, 124.10, 123.00, 120.23, 118.76, 111.50, 106.69, 56.26, 52.45, 49.91,

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46.57, 31.53, 26.82, 26.48, 24.43, 22.64, 14.11. HRMS (EI+) m/z calcd. for $C_{26}H_{28}N_4O_4$ $[M]^+$: 461.2183, found : 461.2209.

2-isopropyl-6-(2-nitrophenyl)-2, 3, 12, 12a-tetrahydropyrazino[1', 2': 1, 6]pyrido[3, 4-b]indole-1, 4(6H, 7H)-dione (3e): Following the general procedure the desired compound **3e** was generated as light yellow solid with yield of 81% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (3:7) as eluent).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.83-7.81 (m, 1H), 7.56 (d, *J* = 6.8 Hz, 1H), 7.48-7.46 (m, 2H), 7.34 (s, 1H), 7.32 (s, 1H), 7.23-7.15 (m, 2H), 4.83 (t, *J* = 6.8, 13.6 Hz, 1H), 4.47 (dd, *J* = 4, 11.6 Hz, 1H), 3.91 (s, 2H), 3.78 (s, 1H), 3.66 (dd, *J*= 4, 15.6 Hz, 1H), 2.99-2.92 (m, 1H), 1.25 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.52, 163.42, 150.57, 136.67, 132.94, 132.61, 130.07, 129.75, 126.26, 124.54, 123.18, 120.38, 118.69, 111.46, 109.23, 54.15, 53.90, 48.12, 44.58, 43.24, 29.40, 27.19, 19.04. HRMS (EI+) m/z calcd. for $C_{23}H_{22}N_4O_4$ $[M]^+$: 419.1714, found: 419.1736. M. P. 86 °C.

6-(2-nitrophenyl)-2-propyl-2, 3, 12, 12a-tetrahydropyrazino[1', 2': 1, 6]pyrido[3, 4-b]indole-1, 4(6H, 7H)-dione (3f): Following the general procedure the desired compound **3f** was generated as yellow solid with yield of 85% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (3:7) as eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.82-7.79 (m, 1H), 7.56 (d, *J* = 8 Hz, 1H), 7.47-7.45 (m, 2H), 7.33 (d, *J* = 5.2 Hz, 2H), 7.23-7.17 (m, 2H), 5.29 (s, 1H), 4.48 (dd, *J*= 3.6, 11.6 Hz, 1H), 4.06 (d, *J* = 16 Hz, 1H), 3.94 (d, *J* = 18 Hz, 2H), 3.66 (dd, *J*= 4, 15.6 Hz, 1H), 3.50-3.44 (m, 2H), 2.99-2.92 (m, 1H), 1.25 (t, *J* = 7.2, 14 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.97, 163.11, 150.53, 136.68, 132.94, 132.65, 130.03, 129.74, 128.91, 126.25, 124.52, 123.18, 120.39, 118.69, 111.48, 109.18, 54.05, 49.31, 48.20, 47.79, 27.33, 19.81, 11.24. HRMS (EI+) m/z calcd. for $C_{23}H_{22}N_4O_4$ $[M]^+$: 419.1714, found : 419.1732. M. P. 82 °C

6-(4,5-dimethoxy-2-nitrophenyl)-2-methyl-2,3,12,12a-tetrahydropyrazino[1', 2': 1, 6]pyrido[3, 4-b]indole-1, 4-(6H, 7H)-dione (3g): Following the general procedure the desired compound **3g** was generated as yellow solid with yield of 79% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (4:6) as eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.60 (d, *J* = 7.6 Hz, 1H), 7.41 (s, 1H), 7.30 (d, *J* = 8 Hz, 1H), 7.21-7.12 (m, 2H), 6.91 (s, 1H), 6.68 (s, 1H), 4.38 (dd, *J*= 4, 11.2 Hz, 1H), 4.13 (d, *J* = 4 Hz, 1H), 4.10 (d, *J* = 7.2 Hz, 1H), 3.92 (d, *J* = 3.2 Hz, 1H), 3.88 (d, *J* = 8 Hz, 1H), 3.85 (s, 3H), 3.75 (s, 3H), 3.27 (dd, *J*=

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16, 12.4 Hz, 1H), 3.07 (s, 3H) ;^{13}C NMR (100 MHz, $CDCl_3$) δ 166.52, 166.32, 153.81, 148.01, 141.37, 136.42, 133.27, 131.72, 126.01, 122.94, 120.15, 118.61, 111.57, 108.47, 107.29, 106.35, 56.46, 56.37, 56.28, 33.92, 24.48. HRMS (EI+) m/z calcd. for $C_{23}H_{22}N_4O_6$ $[M]^+$: 451.1612, found : 451.1635. M. P. 123 °C

6-(4,5-dimethoxy-2-nitrophenyl)-2-benzyl-2, 3, 12, 12a tetrahydropyrazino[1', 2':1, 6]pyrido[3, 4-b]indole-1, 4-(6H, 7H)-dione (3h): Following the general procedure the desired compound **3h** was generated as light yellow solid with yield of 88% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (4:6) as eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.43 (s, 1H), 7.33-7.27 (m, 6H), 7.25-7.14 (m, 2H), 6.95 (s, 1H), 6.70 (s, 1H), 4.74-4.63 (dd, *J* = 4, 11.2 Hz, 1H), 4.47-4.43 (m, 1H), 4.03-3.98 (m, 1H), 3.93-3.92 (m, 1H), 3.89-3.85 (d, *J* = 8 Hz, 1H), 3.87 (s, 3H), 3.72 (s, 3H), 3.41-3.33 (dd, *J* = 16, 12.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.68, 166.47, 153.91, 148.02, 141.09, 136.34, 135.33, 133.59, 131.68, 129.17, 128.46, 128.28, 125.99, 122.97, 120.19, 118.59, 111.58, 108.21, 107.40, 106.17, 56.47, 56.24, 56.20, 52.42, 49.85, 49.60, 29.82, 24.36. HRMS (EI+) m/z calcd. for $C_{29}H_{26}N_4O_6$ $[M]^+$: 527.1925, found : 527.1948.

Representative experimental procedures for the synthesis of hybrids (6a-g). To the stirred solution of compound **3a-g** (1 equiv) in ethanol, sodium dithionate ($Na_2S_2O_4$) (10 equiv.) and potassium carbonate (K_2CO_3) (2 equiv.) were added at 50 ºC under nitrogen atmosphere. After five mintues water was added to dissolve the solid completely and reaction mixture was stirred for thirty mintues. The reaction progress was monitored by thin layer chromatography (TLC), after completion of reaction, the insoluble substance was remove by filtration and filtrate was extracted with ethyl acetate (EtOAc) (3 X 30 mL), dried over sodium sulphate (Na_2SO_4) and evaporated under reduced pressure to obtained reductive product, which was used in next step without further purification.

The crude compound from the previous step was added to a mixture of tetrahydrofuran and acetic acid (1:1) and the resulting solution was stirred for fifteen minutes after which N-bromosuccinimide (NBS) (1 equiv) was added portionwise to stirred reaction mixture. Reaction was monitored by TLC, after completion of which solid $NaHCO_3$ was added to quench the reaction and evaporated under reduced pressure to obtained solid. The solid was extracted with

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ethyl acetate (3 X 30 mL), dried over sodium sulphate and evaporated under reduced pressure to obtain crude compound which was further purified by column chromatography.

3-methyl-2, 3, 4a, 5-tetrahydroindolo[2, 3-b]pyrazino[1', 2':1, 5]pyrrolo[3, 2-c]quinoline-1, 4(10H, 15bH)-dione (6a): Following the general procedure the desired compound **6a** was generated as lemon yellow solid with yield of 70% over two steps *via* flash column chromatography with ethyl acetate-hexane (1:1) as eluent. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8 Hz, 1H), 7.72 (s, 1H), 7.45-7.41 (m, 3H), 7.36 (d, *J* = 8 Hz, 1H), 7.01 (s, 1H), 6.69 (d, *J*= 4 Hz, 1H), 5.53 (s, 1H), 4.92 (m, 1H), 4.31 (d, *J* = 16 Hz, 2H), 4.03 (m, 1H), 3.04 (s, 3H), 2.49 (m, 1H), 2.33 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.42, 167.13, 166.91, 154.91, 142.01, 134.88, 132.96, 130.98, 129.42, 126.86, 123.86, 122.85, 121.42, 120.90, 112.67, 65.67, 61.98, 54.74, 36.68, 33.95, 32.05. M. P. 142 °C

HRMS (EI+) m/z calcd. for $C_{21}H_{18}N_4O_2$ $[M]^+$: 359.1503, found: 359.1506.

3-ethyl-2, 3, 4a, 5-tetrahydroindolo[2, 3-b]pyrazino[1', 2': 1, 5]pyrrolo[3, 2-c]quinoline-1, 4(10H, 15bH)-dione (6b): Following the general procedure the desired compound **6b** was generated as yellow solid with yield of 72% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (1:1) as eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 7.2 Hz, 1H), 7.67 (s, 1H), 7.46-7.34 (m, 4H), 7.04-6.89 (m, 2H), 5.50 (s, 1H), 4.67 (s, 1H), 4.28 (d, *J* = 17.2 Hz, 2H), 4.02 (d, *J* = 16.8 Hz, 1H), 3.50 (d, *J* = 7.2 Hz, 2H), 2.7 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.69, 167.55, 167.17, 156.39, 142.34, 134.85, 132.04, 129.77, 128.92, 127.96, 123.05, 121.30, 119.23, 118.62, 112.11, 65.12, 60.47, 56.47, 51.63, 35.63, 35.29, 29.51, 24.28, 12.61. HRMS (EI+) m/z calcd. for $C_{22}H_{20}N_4O_2$ $[M]^+$: 373.1659, found: 373.1684. M. P. 134 °C

3-benzyl-2, 3, 4a, 5-tetrahydroindolo[2, 3-b]pyrazino[1', 2': 1,5]pyrrolo[3, 2-c]quinoline-1, 4(10H, 15bH)-dione (6c): Following the general procedure the desired compound **6c** was generated as yellow solid with yield of 80% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (1:1) as eluent). ^{1}H NMR (400 MHz, DMSO) δ 11.05 (s, 1H), 7.93-6.90 (m, 13H), 5.52 (d, *J* = 8 Hz, 1H), 4.33 (s, 2H), 3.93 (d, *J* = 15.6 Hz, 1H), 2.09 (d, *J* = 15.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.36, 167.65, 155.09, 141.94, 136.95, 136.60, 135.10, 132.26, 129.90, 128.88, 128.00, 127.44, 126.91, 125.07, 123.31, 119.32, 114.14,

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110.24, 65.82, 65.63, 60.44, 56.41, 51.86, 48.02, 29.50. HRMS (EI+) m/z calcd. for $C_{27}H_{22}N_4O_2$ $[M]^+$: 435.1816, found : 435.1829. M. P. 125 °C

3-hexyl-2, 3, 4a, 5-tetrahydroindolo[2, 3-b]pyrazino[1', 2': 1, 5]pyrrolo[3, 2-c]quinoline-1, 4(10H, 15bH)-dione (6d): Following the general procedure the desired compound **6d** was generated as yellow solid with yield of 65% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (1:1) as eluent). ^{1}H NMR (400 MHz, DMSO) δ 7.79 (d, *J* = 7.6 Hz, 1H), 7.56 (s, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 7.6 Hz, 1H), 6.91 (t, *J* = 8.4, 16.4 Hz, 2H), 5.42 (s, 1H), 4.72-4.68 (m, 2H), 4.34 (d, *J* = 16.8 Hz, 1H), 4.06 (d, *J* = 16.8 Hz, 1H), 3.54-3.47 (m, 2H), 3.15 (t, *J* = 6.4, 13.2 Hz, 2H), 2.69 (s, 2H), 2.56 (s, 6H), 2.2-2.09 (m, 3H) HRMS (EI+) m/z calcd. for $C_{26}H_{28}N_4O_2$ $[M]^+$: 429.2285, found: 429.2311. M. P. 76 °C

3-propyl-2, 3, 4a, 5-tetrahydroindolo[2, 3-b]pyrazino[1', 2':1, 5]pyrrolo[3, 2-c]quinoline-1, 4(10H, 15bH)-dione (6e): Following the general procedure the desired compound **6e** was generated as light solid with yield of 56% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (1:1) as eluent). ^{1}H NMR (400 MHz, DMSO) δ 7.78 (s, 1H), 7.69 (d, *J* = 6.8 Hz, 1H), 7.37 (s, 1H), 7.33 (d, *J* = 8.4 Hz, 1H), 6.93-6.88 (m, 2H), 5.75 (s, 1H), 5.43 (s, 1H), 4.73-4.69 (m, 2H), 4.35 (d ,*J* = 16 Hz, 1H), 4.05 (d, *J* = 16.4 Hz, 1H), 3.51-3.42 (m, 2H), 1.50-1.45 (m, 2H), 0.78 (t ,*J* = 7.2, 14.8 Hz, 3H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.68, 167.68, 167.44, 156.39, 134.85, 129.84, 128.90, 127.97, 123.06, 121.31, 119.24, 118.66, 112.12, 74.58, 65.13, 60.47, 56.48, 52.00, 46.39, 35.32, 20.29, 10.63. HRMS (EI+) m/z calcd. for $C_{23}H_{22}N_4O_2$ $[M]^+$: 387.1816, found : 387.1831. M. P. 95 °C

13,14-dimethoxy-3-benzyl-2, 3, 4a, 5-tetrahydroindolo[2, 3-b]pyrazino[1', 2': 1, 5]pyrrolo[3,2-c]quinoline-1, 4(10H, 15bH)-dione (6f): Following the general procedure the desired compound **6f** was generated as white solid with yield of 80% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (1:1) as eluent). ^{1}H NMR (400 MHz, DMSO) δ 7.64 (d, *J* = 7.6 Hz, 1H), 7.39 (t, *J* = 7.6, 14.8 Hz, 1H), 7.08 (s, 1H), 7.01 (s, 1H), 6.91-6.86 (m, 1H), 5.75 (s, 1H), 4.70-4.65 (m, 1H), 4.05 (d, *J* = 17.2 Hz, 2H), 3.79 (s, 3H), 3.66 (s, 3H), 2.89 (s, 3H), 2.82 (s, 1H), 2.71 (s, 1H) ;^{13}C NMR (100 MHz, DMSO) δ 168.07, 167.62, 167.62, 167.16, 155.68, 148.86, 147.42, 136.89, 134.01, 122.53, 121.73, 119.14, 117.27, 112.03, 110.61, 110.45, 65.19, 61.35, 56.20, 55.66, 55.52, 53.85, 35.74, 33.01. HRMS (EI+) m/z calcd. for $C_{29}H_{26}N_4O_4$ $[M]^+$: 495.2027, found : 495.2058. M. P. 103 °C

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Representative procedure for the synthesis of hybrids 4a-d. Compound **3a-c** and **g** (1 equiv) in a mixture of THF:AcOH:H_2O (1:1:1) and was stirred for fifteen minutes and then N-bromosuccinimide(1 equiv) was added portion-wise to stirred reaction mixture and continue stir for 3h. Reaction was monitored by TLC, after completion of reaction solid sodium bicarbonate ($NaHCO_3$) was added to quench the reaction and evaporated under reduced pressure to obtaina solid residue. The solid was dissolvedinEtOAc, dried over sodium sulphate (Na_2SO_4) and evaporated under reduced pressure to obtained crude compound which was further purified by column chromatography.

(R)-1-methyl-3-((2-(2-nitrobenzoyl)-1H-indol-3-yl)methyl)piperazine-2 , 5-dione (4a): Following the general procedure the desired compound **4a** was generated as white gummy liquid with yield of 82% (purified *via* flash column chromatography with ethyl acetate-hexane (1:1) as eluent). ^{1}H NMR (400 MHz, DMSO) δ 7.97 (d, *J* = 3.2 Hz, 1H), 7.95 (s, 1H), 7.81 (s, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.29 (t, *J* = 7.2, 14.8 Hz, 1H), 7.24 (s, 1H), 7.09 (t, *J* = 7.2, 14.4 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H), 3.50 (d, *J* = 17.2 Hz, 1H), 3.40-3.35 (m, 2H), 2.88 (s, 3H), 2.51 (s, 2H) ; ^{13}C NMR (100 MHz, DMSO) δ 166.00, 165.28, 162.30, 153.82, 149.26, 138.40, 136.89, 131.98, 130.36, 127.47, 125.89, 121.00, 120.09, 117.59, 112.71, 110.69, 107.31, 56.60, 56.37, 56.62, 50.51, 35.78, 32.92, 30.77. HRMS (EI+) m/z calcd. for $C_{21}H_{18}N_4O_5$ $[M]^+$: 407.1350, found: 407.1372. M. P. 204 °C

(R)-1-ethyl-3-((2-(2-nitrobenzoyl)-1H-indol-3-yl)methyl)piperazine-2, 5-dione (4b): Following the general procedure the desired compound **4b** was generated as colorless oil with yield of 80% (purified *via* flash column chromatography with ethyl acetate-hexane (1:1) as eluent). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.23-8.19 (m, 1H), 7.81-7.75 (m, 1H), 7.72-7.67 (m, 3H), 6.32 (s, 1H), 4.35 (s, 1H), 3.86-3.81 (m, 1H), 3.45 (dd, *J* = 8, 16 Hz, 1H), 3.29-3.24 (m, 1H), 3.11-3.04 (m, 2H), 0.87 (t, , *J* = 4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.66, 165.72, 165.25, 146.34, 136.91, 135.47, 134.83, 131.22, 129.30, 127.98, 127.70, 125.04, 121.86, 121.81, 119.72, 112.38, 56.84, 48.40, 41.17, 29.47, 11.46. HRMS (EI+) m/z calcd. for $C_{22}H_{20}N_4O_5$ $[M]^+$: 421.1506, found : 421.1529. M. P. 175 °C

(R)-1-benzyl-3-((2-(2-nitrobenzoyl)-1H-indol-3-yl)methyl)piperazine-2, 5-dione (4c): Following the general procedure the desired compound **4c** was generated as colorless oil with yield of 80% (purified *via* flash column chromatography with ethyl acetate-hexane (1:1) as

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eluent).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 8.22 (d, *J* = 8 Hz, 1H), 7.78-7.76 (m, 2H), 7.67 (d, *J* = 7.6 Hz, 2H), 7.41-7.32 (m, 3H), 7.24-7.19 (m, 3H), 7.03-7.00 (m, 2H), 6.29 (s, 1H), 4.63 (d, *J* = 14.4 Hz, 1H), 4.46 (s, 2H), 3.99 (d, *J* = 14.4 Hz, 1H), 3.90 (dd, *J* = 4.8, 14 Hz, 2H), 2.92 (d, *J* = 18 Hz, 1H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.64, 165.61, 165.39, 146.35, 134.88, 134.78, 131.22, 129.26, 128.99, 128.49, 128.18, 127.76, 125.03, 121.89, 121.84, 112.47, 56.74, 49.63, 48.33, 29.84, 29.60. HRMS (EI+) m/z calcd. for $C_{27}H_{22}N_4O_5$ $[M]^+$: 483.1663, found: 483.1689. M. P. 167 °C

(R)-3-((2-(4, 5-dimethoxy-2-nitrobenzoyl)-1H-indol-3-yl)methyl)-1-methylpiperazine-2, 5-dione (4d): Following the general procedure the desired compound **4d** was generated as colorless oil with yield of 71% (purified *via* flash column chromatography with ethyl acetate-hexane (1:1) as eluent). ^{1}H NMR (400 MHz, DMSO) δ 7.97 (d, *J* = 3.2 Hz, 1H), 7.95 (s, 1H), 7.81 (s, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.29 (t, *J* = 7.2, 14.8 Hz, 1H), 7.24 (s, 1H), 7.09 (t, *J* = 7.2, 14.4 Hz, 1H), 3.97 (s, 3H), 3.90 (s, 3H), 3.50 (d, *J* = 17.2 Hz, 1H), 3.40-3.35 (m, 2H), 2.88 (s, 3H), 2.51 (s, 2H) ; ^{13}C NMR (100 MHz, DMSO) δ 166.00, 165.28, 162.30, 153.82, 149.26, 138.40, 136.89, 131.98, 130.36, 127.47, 125.89, 121.00, 120.09, 117.59, 112.71, 110.69, 107.31, 56.60, 56.37, 56.62, 50.51, 35.78, 32.92, 30.77. HRMS (EI+) m/z calcd. for $C_{23}H_{22}N_4O_7$ $[M]^+$: 467.1561, found: 467.1592. M. P. 109 °C

Representative experimental procedures for the synthesis of hybrids (5a-c). To the stirred solution of compound **2a-c** (1 equiv) in ethanol, sodium dithionate ($Na_2S_2O_4$) (10 equiv.) and potassium carbonate (K_2CO_3) (2 equiv.) were added at 50 ºC under nitrogen atmosphere. After five mintues water was added to dissolve the solid completely and reaction mixture was stirred for thirty mintues. The reaction progress was monitored by thin layer chromatography (TLC), after completion of reaction, the insoluble substance was remove by filtration and filtrate was extracted with ethyl acetate (EtOAc) (3 X 30 mL), dried over sodium sulphate (Na_2SO_4) and evaporated under reduced pressure to obtained reductive product, which was used in next step without further purification.

The crude compound from the previous step was added to a mixture of tetrahydrofuran and acetic acid (1:1) and the resulting solution was stirred for fifteen minutes after which N-bromosuccinimide (NBS) (1 equiv) was added portion wise to stirred reaction mixture. Reaction was monitored by TLC, after completion of which solid $NaHCO_3$ was added to quench the

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reaction and evaporated under reduced pressure to obtained solid. The solid was extracted with ethyl acetate (3 X 30 mL), dried over sodium sulphate and evaporated under reduced pressure to obtain crude compound which was further purified by column chromatography.

Methyl 2, 3, 8, 13b-tetrahydro-1H-indolo[2, 3-b]pyrrolo[3, 2-c]quinoline-2-carboxylate (5a). Following the general procedure the desired compound **5a** was generated as colorless gummy solid with yield of 64% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (6:4) as eluent).^{1}H NMR (400 MHz; $CDCl_3$): 7.81(s,1H);7.43-7.41 (d, *J* = 8Hz, 1H); 7.37-7.34 (dd, *J* = 4Hz, 8Hz,1H); 7.29-7.25 (m,2H); 7.16-7.14 (d, *J* = 8 Hz, 1H); 7.04-7.01 (m, 1H); 6.94-6.92 (d, *J* = 8Hz, 1H); 4.67 (s,1H); 4.53(s, 1H); 3.91-3.87(m, 1H); 3.75(s, 3H); 2.70-2.63(m, 2H); 2.12-2.07(m, 1H). M. P. 72 °C

Methyl-11-chloro-2, 3, 8, 13b-tetrahydro-1H-indolo[2, 3-b]pyrrolo[3, 2-c]quinoline-2-carboxylate (5b). Following the general procedure, the desired compound **5b** was generated as light yellow oil with yield of 57% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (7:3) as eluent). ^{1}H NMR (400 MHz; $CDCl_3$): 7.55-7.51(t,*J*=8Hz, 2H); 7.43-7.41(t, *J* = 8 Hz 1H); 7.37-7.35(d, *J* = 8 Hz, 1H); 7.29-7.23 (m, 3H); 4.75 (s, 1H); 4.35-4.30 (m, 1H); 3.61(s, 3H); 2.67-2.62 (m, 1H); 2.27-2.22(m,1H). ^{13}C NMR (100 MHz; $CDCl_3$): 173.18, 170.65, 140.49, 135.39, 133.88, 132.77, 130.05, 129.94, 126.91, 125.45, 123.01, 122.87, 119.37, 113.95, 113.95, 61.29, 57.29, 55.19, 52.72, 39.63. $[M+H]^+$ calculated for ($C_{19}H_{16}N_3ClO_2$) 354.1044 found 354.1028. M. P. 86 °C

Methyl-12-chloro-2, 3, 8, 13b-tetrahydro-1H-indolo[2, 3-b]pyrrolo[3, 2-c]quinoline-2-carboxylate (5c). Following the general procedure, the desired compound **5c** was generated as light yellow oil with yield of 75% over two steps (purified *via* flash column chromatography with ethyl acetate-hexane (7:3) as eluent). ^{1}H NMR (400 MHz; $CDCl_3$): 7.49(s,1H); 7.43-7.41(d, *J* = 8Hz,1H); 7.32-7.28(t, *J* = 8 Hz, 1H); 7.19-7.14 (m,2H); 7.07-7.04 (t, *J* = 8 Hz, 1H); 6.98-6.96 (d, *J* = 8 Hz, 1H); 4.58 (s, 1H); 4.29-4.24 (m, 1H); 3.62 (s, 3H); 3.50-3.45 (m, 1H); 2.55-2.50 (m, 1H). ^{13}C NMR (100 MHz; $CDCl_3$): 173.86, 171.36, 146.79, 136.89, 135.25, 129.18, 129.02, 128.89, 128.57, 126.52, 122.81, 122.20, 121.03, 113.86, 62.04, 57.70, 54.45, 52.46, 39.27. $[M+H]^+$ calculated for ($C_{19}H_{16}N_3ClO_2$) 354.1004, found 354.1037. M. P. 84 °C

Biological assays

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Cell culture

MCF-7 cell line derived from pleural effusion of breast adenocarcinoma from a female patient was grown in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen Bioservices India Pvt. Ltd.) supplemented with 10% Fetal Bovine Serum (FBS, Gibco, USA) with 1% antibiotics (pencillin-10,000 units/ml, Streptomycin-10,000 μg/ml, Gibco, USA). Cells were confluent at the time of experimentation (Cell confluency ~ 80%), then cells were dislodged from flasks by trypsinization containing 0.25% Trypsin. All cell lines were purchased from NCSS (www.nccs.res.in)

Compound exposure for dose-response curves

15000 cells/200 μl of media were plated per well in 96-well plates and were allowed to adhere for 18 hours. Adhere cells were then treated with 1-100 μg/ml compounds **3a**, **b**, **e-f**, **4g**, **6a-g**, **5a** and Doxorubicin in triplicates for 24 hours. All the solutions were prepared from concentrated stock solutions (in DMSO) of the compounds.

Viability assays

After 24 hours of cell incubation in the presence or absence of each compound, cell viability was evaluated by using MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)) (Sisco Research Laboratories Pvt. Ltd., India). In brief, this is homogeneous, colorimetric method for determining the number of viable cells in proliferation, cytotoxicity or chemosensitivity assays. MTT is bioreduced by cells into a formazan product that is soluble in tissue culture medium. After 24 hours of compound treatment media was removed and 100μl of MTT (0.5 mg/ml in media) was added to the cells and was kept in dark for 2 hours at 37^{o}C and resulting formazan formed was dissolved in 100μl DMSO (Dimethyl Sulfoxide, ACS).[14-15] The absorbance of the formazan product at 595 nm was measured directly from 96-well assay plates without additional processing by a multimode Plate reader (Bio-Rad) (iMark, India), as absorbance is directly proportional to the number of viable cells in culture. Percentage of viable cells in each group is determined with respect to untreated control cells.

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